An atlas of organ microbiomes of *Biomphalaria* spp., the snail host of schistosome parasites

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INTRODUCTION

The **microbiome** – the microorganism community that inhabits on or within an animal's body – is increasingly **recognized to shape** many **aspects** of its **host biology** and is a **key determinant of health and disease** [1,2], notably by increasing or reducing **susceptibility to parasites** [3,4]. Many **mollusks** and **crustaceans** are known to **harbor bacteria in their hemolymph** (*i.e.* blood [5]) and **bacteria** are **known to be present** in ***Biomphalaria* snails** from classical bacterial culture work in the 1980s [6] although the **hemolymph microbiome** from these snails remain **poorly characterized.**

The central aim of this study is to **pioneer research** on the **microbiome of freshwater snails** from the genus ***Biomphalaria* spp.** which are **intermediate host** for the human blood fluke **parasite *Schistosoma mansoni***, causative agent of **schistosomiasis**, a parasitic disease that infects over **67 million people** in **sub-Saharan Africa** and **South America**. We previously sequenced the bacterial 16S ribosomal DNA from the **hemolymph (blood)** of *Biomphalaria* spp. Snails [6] revealing a **diverse microbiome**. We hypothesized that the snail microbiome may represent a **critical**, but **unexplored intermediary** in the **snail-schistosome interaction** as the parasite is in very close contact with organs and hemolymph during its development.

To understand the heterogeneity of the microbiome in different snail organs bathed by the hemolymph, we sampled **4 organs** (ovotestis, liver, gut, and stomach), and the **hemolymph** from **five snails** from **two different species** (*B. alexandrina* and *B. glabrata*).These results will pave the way for further investigations into the role of microbiome on both snail biology and schistosome infections.

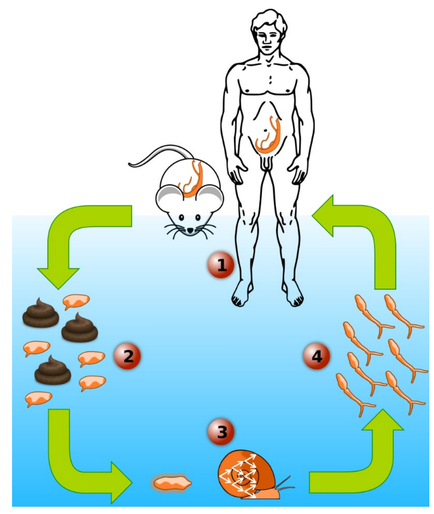


Fig. 1. - <u>*Schistosoma mansoni* life cycle.</u> (1) Male and female adult worms are found in human portal vein. Fertilized female worms migrate in the venules draining the intestine to lay eggs which then pass through the intestine wall to the lumen. **(2)** Eggs are excreted with feces and hatch in fresh water. **(3)** Motile miracidia actively seek the snail intermediate host, penetrate the body wall, differentiate into sporocysts, and proliferate asexually during a month. **(4)** One month after infection, snails release motile clonal cercariae into the water. Cercariae penetrate the unbroken skin of a mammalian host.

MATERIALS AND METHODS

Snail anatomy

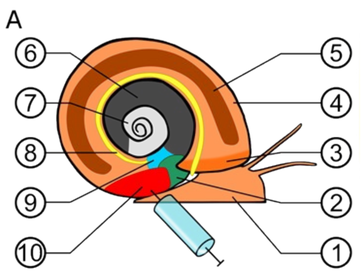
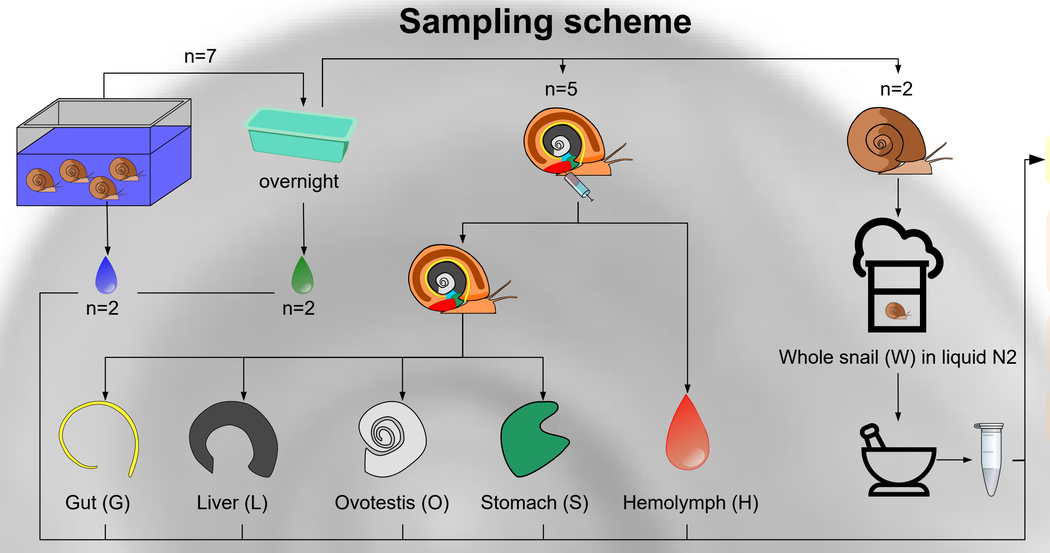
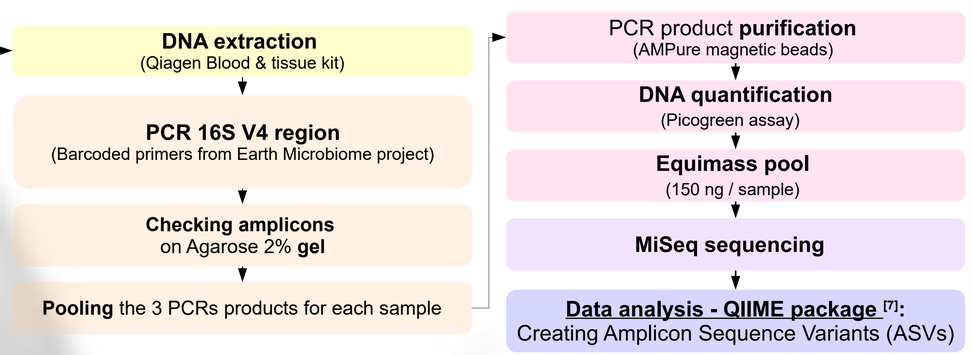


Fig. 2. - <u>Simplified snail anatomy.</u> 1: head foot; **2:** albumen gland; **3:** mantle cavity; **4:** mantle; **5:** kidney; **6:** digestive gland (liver); **7:** reproductive gland (ovotestis); **8:** gut; **9:** stomach; **10:** heart with a syringe showing the puncture site.

Sampling scheme



Sample processing



RESULTS

Sequencing effort

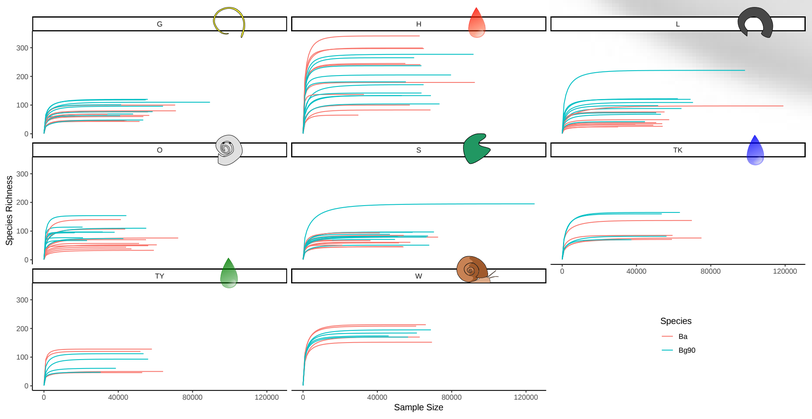


Fig. 3. - <u>Rarefaction curves based on species richness for all the samples analyzed.</u> The plateau phase obtained for all the samples demonstrated that we have **sequenced deep enough to capture all microbial diversity.**

Taxonomic diversity

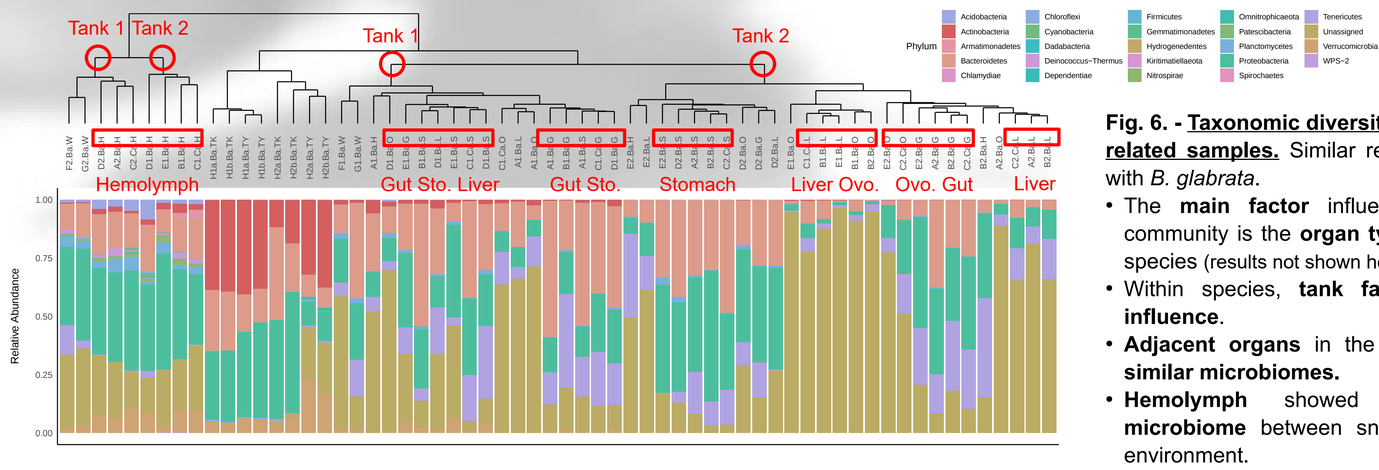


Fig. 6. - <u>Taxonomic diversity in *B. alexandrina* related samples.</u> Similar results were obtained with *B. glabrata*.

- The **main factor** influencing the bacterial community is the **organ type**, before the snail species (results not shown here).
- Within species, **tank factor** has a **major influence**.
- **Adjacent organs** in the snail tend to have **similar microbiomes.**
- **Hemolymph** showed relatively **stable microbiome** between snails from the same environment.

Microbial alpha diversity: diversity within samples

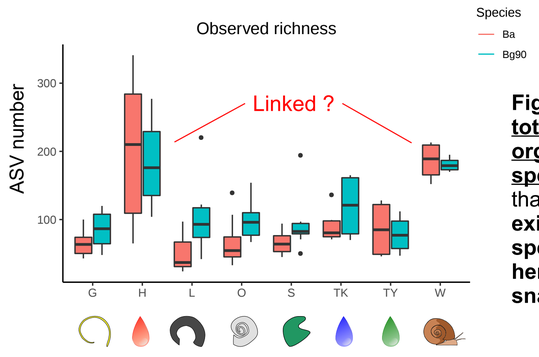


Fig. 4. - <u>Distribution of the total number of ASVs per organ or water for each snail species.</u> We demonstrated that **organ microbiomes exist**. Moreover, the **highest species richness** is found in **hemolymph** and in **whole snail**.

Microbiome sharing between organs (qualitative = absence/presence)

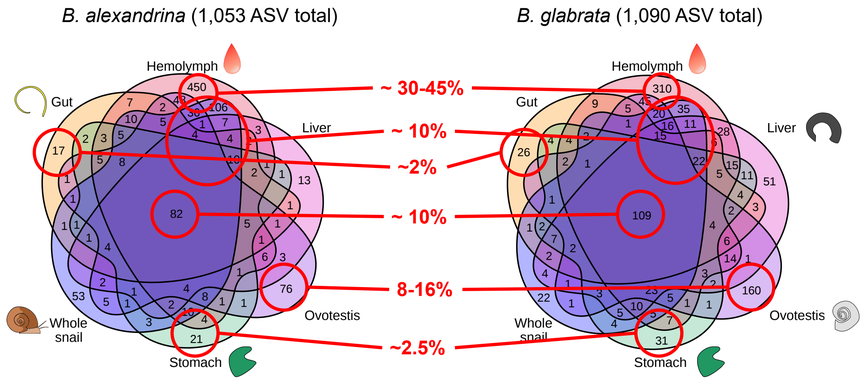


Fig. 7. - <u>Venn diagrams highlighting microbiome taxa sharing between organs for each snail species.</u>

- **Most** of the **taxa** are **specific** to the **hemolymph**.
- **Limited** number of **taxa shared** between **hemolymph** and **whole snail.**
  => **whole snail microbiome** gives a **poor representation** of the **hemolymph microbiome.**
- **Low** number of **taxa shared across all organs**
  => **microbiome** relatively **partitioned**
- **Ovotestis** is the **organ** showing the **2nd highest** number of **specific taxa**
  => role in **egg development**?
- **Stomach** and **gut** show **limited taxa** diversity.

CONCLUSION

We found that each organ harbors its own microbiome. These microbiomes showed significant differences in composition from different snail organs and hemolymph. The **highest microbial diversity** is found in **the hemolymph and the whole snails** while the other organs showed more limited diversity. **Sample type** (organs and hemolymph), rather than snail species, is the **main factor** explaining the difference in **microbial diversity**, suggesting that specific organ environment shapes its microbiota. Furthermore, the physical distance between organs reflects the relative distance between organ microbiomes.

These results suggest that **sampling hemolymph** may be **sufficient to capture most of the microbial diversity** present in snails but sampling individual organs can provide a more complete description of the snail microbiome. Sampling **microbiomes from whole snails** provides a **composite microbiome from several organs and tissues** so may not be ideal.

<u>References</u>: [1] Kinross *et al.,* 2011; Genome Med. - **[2] Oliver *et al.,* 2010**; Ann. Rev. Entomol. - **[3] Slatko *et al.*, 2014;** Mol. Biochem. Parasitol. - **[4] Sant'Anna *et al.*, 2014;** Parasit. Vectors – **[5] Desriac *et al.,* 2014;** FMES Microbiol. Lett. - **[6] Chevalier *et al.*, 2020**; Environmental Microb. - **[7] Caporaso *et al.*, 2010**; Nature Methods